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Enhanced extraction of procyanidins from avocado processing residues by pulsed electric fields pre-treatment

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ABSTRACT

The waste generated by the global production of avocado (*Persea americana* Mill), which includes peels, seeds, and pulp, poses environmental challenges. The principles of circular economy offer a sustainable solution by valorizing this waste and transforming it into valuable products. This study investigates the potential of avocado by-products, particularly peels and seeds, as a source of procyanidins. Pulsed electric field (PEF) technology has been shown to increase the extraction yield of valuable compounds from food waste/losses. In our study, we optimized the PEF treatment of avocado peels and seeds for the extraction of procyanidins by using Box-Behnken designs with response surface methodology and desirability functions. The effects evaluated were the frequency (1, 50, 100 Hz), the electric field strength (0.8, 1.1, 1.5 kV/cm) and the number of pulses (100, 150, 200) for avocado peel, while for the avocado seeds were the frequency (50, 100, 150 Hz), the electric field (0.6, 0.7, 0.8 kV/cm) and the total operating time (1, 5, 10 s). Our study showed that the procyanidin content measured by HPLC-FLD significantly increased in PEF-pretreated avocado peel and seeds. The optimized treatment conditions include a frequency of 50 Hz, an electric field strength of 1.31 kV/cm, and 175 electric pulses for the avocado peels, resulting in a procyanidin content of 9798.32 ± 103.83 μg CE/g d.w. For avocado seeds, the optimal conditions include a frequency of 100 Hz, an electric field strength of 0.75 kV/cm, and total operating time of 5.5 s, resulting in a procyanidin content of 21103.12 ± 234.76 μg/g d.w. The precision and validity of the mathematical models confirmed the reliability of these optimal conditions. These results emphasize the potential of PEF technology for the extraction of procyanidin from avocado waste and contribute to environmentally friendly extraction techniques in the food industry.

1. Introduction

Avocado (*Persea americana* Mill) is a fruit native to the tropical regions of America. It is classified under the *Persea* (Clus.) Miller genus within the *Lauraceae* family (Jimenez et al., 2021). The *Lauraceae* family stands as one of the earliest among flowering plants. Avocado holds significant importance within this family as the primary edible fruit, boasting considerable commercial worth. Initially concentrated in Central and South America, its cultivation has now expanded globally (García-Vargas, Contreras, & Castro, 2020). Avocado consists mainly of the flesh (65–73%), the seed (16–20%) and the skin (11–15%). Avocado is predominantly eaten fresh, but the increasing global demand for its production and processing has led to a surge in avocado waste. When avocado by-products - such as peel and seeds - are discarded during processing, they cause environmental issues such as greenhouse gas emissions, soil and water pollution, and the attraction of pests. This waste poses urgent environmental challenges, with an estimated 40% of total avocado production being wasted or discarded as by-products (Sandoval-Contreras, González Chávez, Poonia, Iñiguez-Moreno, & Aguirre-Güitrón, 2023). To tackle these challenges, the valorization of avocado waste is crucial for sustainable development. It offers the opportunity to transform waste into valuable products while limiting the

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environmental impact (Galanakis, 2012). Taking into account the principles of circular economy, this approach ensures efficient resource use and waste reduction, thereby lessening the detrimental environmental impacts associated with avocado waste disposal (Araújo, Rodriguez-Jasso, Ruiz, Pintado, & Aguilar, 2018). At the same time, it opens doors for economic growth by providing alternative raw materials for value-added products (Galanakis, 2013). In recent years, avocado by-products have received increased attention due to their potential as sources for bioactive compounds like phenolics, carotenoids, and sterols. So far, avocado by-products have been used in producing biofuels, biodegradable plastics, animal feed, natural fertilizers, as a substrate for the growth of mushrooms and microorganisms, and as food ingredients and supplements (Sandoval-Contreras et al., 2023). In particular, the phenolic compounds known as procyanidins or flavan-3-ols from avocado peels and seeds have been attributed several bioactivities, such as in vitro antimicrobial activity against *Helicobacter pylori, Staphylococcus* spp. and *Candida* spp., as well as cytotoxicity, anti-inflammatory and antidiabetic activities. In vivo studies have also shown the hypoglycemic and tissue-protective effects of hydroalcoholic procyanidins extracts from peel and seed on pancreas, kidneys, and liver in alloxan-induced albino rats. In food sector, ethanolic extracts from avocado seeds rich in procyanidin have shown a protective effect against the fatty acid degradation in sunflower oil (Jimenez et al., 2021).

Therefore, to efficiently recover phytoconstituents from avocado peels and seeds could benefit various industries such as biotechnology, pharmaceuticals, food, and cosmetics. The critical steps involve optimizing extraction procedures, quantifying the isolated extracts, ensuring their stability, and preserving their bioactivity during storage. In extraction, the use of novel emerging extraction technologies can offer advantages such as mitigating environmental impact, reducing energy consumption, minimizing the use of organic solvents, and shortening extraction times compared to conventional methods. The application of these efficient extraction methods will be beneficial for the recovery of bioactives from fruit and vegetable waste (Rifna, Misra, & Dwivedi, 2023).

Previously, phenolic compounds from avocado wastes have been reported to be extracted by different technologies such as hydroethanolic liquid-liquid extraction (Melgar et al., 2018), ultrasound assisted (Monzón, Becerra, Aguirre, Rodríguez, & Villanueva, 2021; Razola-Díaz, Verardo, Guerra-Hernández, García-Villanova Ruiz, & Gómez-Caravaca, 2023; Segovia, Corral-Pérez, & Almajano, 2016; Tremocoldi et al., 2018), supercritical fluid extraction (Restrepo-Serna, Solarte-Toro, & Cardona-Alzate, 2022), subcritical water extraction (Mazyan et al., 2021), microwave (Araujo et al., 2021; Carvalho Gualberto et al., 2021), and vacuum microwave (Skenderidis, Leontopoulos, Petrotos, & Giavasis, 2021) assisted extractions. Another technology, pulsed electric fields (PEF), is one of the most widely recognized emerging technology that has been shown to improve drying, diffusion, and extraction processes. In PEF extraction, short pulses of moderate to high electric field strengths, and relatively low energy (1–10 kJ/kg) are applied to products located between two electrodes in a batch or continuous treatment chamber, usually at or slightly above room temperature. The treatment chamber, which contain the products and two electrodes separated by an insulating material, is connected to a pulse generator consisting of a charger that converts alternating current into direct current, and charging an energy storage device such as a capacitor or an inductor (Raso et al., 2016; Razola-Díaz, Tylewicz, Rocculi, & Verardo, 2023). Combining PEF as a pre-treatment followed by ultrasound extraction can offer several advantages for extracting phenolic compounds from plant materials as previously reported by other authors (Martín-García et al., 2020; Ntourtoglou et al., 2022; Tzima, Brunton, Lyng, Frontuto, & Rai, 2021). Firstly, PEF pre-treatment primes the plant material for more efficient extraction by disrupting cell membranes, making them more permeable to solvent penetration. This initial step enhances the accessibility of intracellular phenolic compounds, setting the stage for the subsequent ultrasound extraction process (Martín-García et al., 2020). The synergistic effect of PEF and ultrasound leads to higher extraction yields compared to using either method alone. While PEF facilitates initial cell membrane permeabilization, ultrasound further disrupts cell structures and enhances mass transfer, ensuring more thorough extraction of phenolic compounds. This combined approach maximizes the extraction efficiency and improves overall yield (Ntourtoglou et al., 2022). Furthermore, the use of PEF as a pre-treatment followed by ultrasound extraction can reduce processing time. PEF facilitates rapid cell membrane permeabilization, allowing the cavitation caused by the ultrasounds and the solvent used to increase diffusion of the solvent in the plant tissue and accelerate the extraction process. The shortened processing time enhances productivity and efficiency in phenolic compound extraction, making the overall process more time-effective (Tzima et al., 2021). Besides, the scalability and eco-friendliness of both PEF and ultrasound technologies make them suitable for various production scales and environmentally sustainable extraction processes (Arshad et al., 2021). With minimal or no chemical solvents required, the combined PEF-ultrasound extraction process offers a greener alternative to traditional solvent-based extraction methods, aligning with the growing demand for sustainable and eco-friendly extraction techniques (Chakka, Sriraksha, & Ravishankar, 2021; Tylewicz, 2020).

PEF pre-treatment have previously been used to extract phenolic compounds from several matrices such as grape by-products (Barba, Brianceau, Turk, Boussetta, & Vorobiev, 2015; Brianceau, Turk, Vitrac, & Vorobiev, 2015; Corrales, Toepfl, Butz, Knorr, & Tauscher, 2008; Medina-Meza & Barbosa-Cánovas, 2015), blueberry by-products (Bobinaitė et al., 2015), papaya peels and seeds (Parniakov, Barba, Grimi, Lebovka, & Vorobiev, 2014, 2015), plum peels (Medina-Meza & Barbosa-Cánovas, 2015), mango peels (Parniakov, Barba, Grimi, Lebovka, & Vorobiev, 2016), range peels (Luengo, Álvarez, & Raso, 2013), and olive kernels (Roselló-Soto et al., 2015), among others. However, this technology have never been applied to extract phenolic compounds from avocado wastes.

The aim of our work was to optimize the conditions for PEF as a pretreatment to ultrasonic extraction of valuable procyanidins from avocado peels and seeds. The procyanidin content and profile were analyzed by liquid chromatography (HPLC-FLD-DAD). The optimization was performed by using Box Behnken designs and quadratic desirability functions. The Box-Behnken design is a response surface methodology used for experiments with multiple variables. A quadratic desirability function complements this by allowing simultaneous optimization across these variables, enabling to find optimal conditions for procyanidin extraction.

2. Materials and methods

2.1. Samples and reagents

Catechin standard was purchased from Sigma-Aldrich (St. Louis, MO, USA). Double-deionized water was obtained from Millipore (Bedford, MA, USA). HPLC-grade water, acetic acid, acetonitrile, and methanol were purchased from Merck KGaA (Darmstadt, Germany).

Avocado by-products of the 'Hass' variety were obtained from a local company located in the subtropical coast of the province of Málaga (Vélez-Málaga, Spain) in April 2021. The by-products from the production of guacamole, peels (moisture ~75%) and seeds (moisture ~45%), were provided separately.

Managing the large amounts of by-products generated industrially can be challenging due to their high moisture content, which makes them prone to spoilage (Cheng, Chiu, & Lo, 2017). Over time, the phenolic compounds in the by-products can also degrade due to enzyme activity. We encountered the same issue in the lab, as we were not able to process the by-products on the same day they were received. Additionally, we aimed to avoid variations in treatment performance that could result from differences in moisture content between samples.

Therefore, we used the by-products in dried form. The peels were dried (as previously optimized) at 40 °C in a hot air dryer with an airflow of 1.6 m/s for 105 min (final moisture <10%) (Razola-Díaz, Guerra-Hernández, Gómez-Caravaca, García-Villanova, & Verardo, 2023). The seeds were dried for longer time (98 h) at the same temperature until they reached a final moisture <10%. The samples were stored at −18 °C until analysis.

2.2. Experimental design

The process to obtain avocado peels and seeds extracts enriched in procyanidins has been optimized using Box-Behnken designs. Each design consisted in 15 experiments with three levels (−1, 0, 1) carried out in duplicate. The independent factors in the case of avocado peels were the frequency (1, 50, 100 Hz), the electric field strength (0.8, 1.1, 1.5 kV/cm) and the number of pulses (100, 150, 200), while for the avocado seeds were the frequency (50, 100, 150 Hz), the total operating time (1, 5, 10 s) and the electric field (0.6, 0.7, 0.8 kV/cm). The dependent variable was total procyanidins expressed in μg catechin equivalents (CE)/g dry weight (d.w.). The variables were fitted to a second order polynomial equation (Equation (1)) where Υ represents the response variable, X_i and X_j are the independent factors that affect the response and β_0, β_i, β_{ii} and β_{ij} are the regression coefficients of the model (interception, linear, quadratic and interaction terms, respectively).

Equation (1). Second order polynomial equation.

$$\Upsilon = \beta_0 + \sum_{i=0}^{4} \beta_i X_i + \sum_{i=0}^{4} \beta_{ii} X_{ii}^2 + \sum_{i=0}^{4} \sum_{j=0}^{4} \beta_{ii} X_i X_j \tag{1}$$

In order to evaluate the adjustment of the models, ANOVAs analyses were performed. Response surface methodology (RSM) was used for establishing the optimal conditions linked to desirability quadratic functions. The software used for the simulations and statistical analysis was Statistica 8.0 package (StatSoft, Tulsa, OK, USA).

2.3. Pulsed electric field treatment

PEF pre-treatments were performed using a lab-scale PEF unit delivering a maximum output voltage and current of 8 kV and 60 A, respectively (mod. SP7500, Alintel srl., Bologna, Italy). The PEF generator used provided monopolar rectangular-shape pulses and adjustable pulse duration (5–20 μs), and pulse frequency (50–500 Hz). The pulse width was fixed to 10 μs for all treatments. The chamber size consisted into parallel stainless-steel electrodes with a 2 cm gap for the avocado peel and 1.8 cm gap for the avocado seed in static mode. The initial temperature was ≈20 °C and the final temperature after treatment was ≤40 °C in all cases. Peels were rehydrated for 20 min and treated in pieces of 2–3 cm (3 g for each treatment) in tap water with a liquid initial conductivity of 0.21 mS/cm (measured by EC-Meter basic 30+, Crison). Otherwise, avocado seeds were grounded, and a paste was prepared at a ratio of 1:2 avocado seeds:tap water, used in doses of 25 mL for each treatment with an initial conductivity of 4.26 mS/cm.

The specific treatment conditions applied for each sample were given by the Box Behnken experimental designs.

The specific energy (kJ/kg) for each treatment was calculated as follow:

$$\textit{Specific energy}\left(\frac{kJ}{kg}\right) = \frac{\textit{Voltage }(V)\textit{ x pulse width }(s)\textit{ x Current }(A)}{\textit{Volume of treatment chamber }(L)\textit{ x density}\left(\frac{kg}{L}\right)}$$

After the PEF treatment the samples were subjected to freeze-drying (Thermo HETO, powerdry LYOLAB 3000; Waltham, USA) and kept at −18 °C till the extractions.

2.4. Ultrasound assisted extraction

For each experiment 0.5 g of sample was weighted and 100 mL of ethanol/water (v/v) was added in 250 mL beakers. They were submitted to extraction for 30 min by using a lab sonicator (UP400St ultrasonic processor, Hielscher, Germany) of 400W and 24 kHz, equipped with an ultrasonic probe of 22 mm of diameter with conditions previously optimized (Razola-Díaz, Verardo, et al., 2023). For the peels and seeds it was used ethanol at the concentration of 60% and 55%, respectively. Moreover, the amplitude of 70% and 90% was used for peel and seeds, respectively. The pulse was locked at 100% for all the analysis. The specific ultrasonic power applied for each sample were 2800 W/L for the peels and 3600 W/L for the seeds. After the extraction, the content of the beaker was transferred to a Falcon tube, and centrifugated at 9000 rpm for 10 min. The supernatants were collected, evaporated using a rotavapor (R-100 Buchi, Barcelona, Spain), and reconstituted in 2–4 mL of methanol/water (1:1, v/v). The final extracts were filtered using cellulose acetate filters of 0.2 mm (Millipore, Bedford, MA, USA) and stored at −18 °C until analyses.

2.5. Determination of procyanidins by HPLC-FLD

The determination of procyanidins in avocado samples was carried out by a methods previously described with slight modifications (Hollands et al., 2017). The extracts were analyzed by high liquid performance chromatography (HPLC) in an Agilent 1200 Series (Agilent Technologies, Palo Alto, CA, USA) equipped with a quaternary pump delivery system, a degasser, an autosampler and a fluorescence detector (FLD). The final selected column was Luna Hilic column (150 × 2.0 mm; 3 μm) (Phenomenex, Torrance, CA, USA). The mobile phase consisted of 98% acetonitrile and 2% acetic acid (A) and 95% methanol, 3% water and 2% acetic acid (B). Samples were eluted with an increasing gradient of (B): 0 min,7%; 3 min, 7%; 15 min, 30%; 40 min, 49%; 40.1 min, 7%; and 55 min, 7% at a flow rate of 0.350 mL/min. The total run time was 57 min, the mid-peak RT of dp 10 being 28 min. The injection volume was 2 μL. The column temperature was held at 35 °C. The fluorescence detector wavelengths were 290 nm for excitation and 325 nm for emission. To ensure that all analytes were within the linear range of the detector, the photomultiplier tube gain was set to 9. Monomeric content of the extracts was calculated in relation to a response factor derived from a catechin standard curve over the range 0–100 μg/mL. Oligomeric procyanidins (dp2-10) were calculated using the relative fluorescence response factors previously established by Hollands et al. (2017)).

3. Results and discussion

3.1. Fitting the model

When biological cells are exposed to an external electric field, the accumulation of charge on their membrane surfaces triggers an increase in transmembrane potential, leading to the formation of pores, either reversible or irreversible, in weaker areas of the membrane. This electroporation significantly increases membrane permeability, potentially resulting in cell disintegration. Electroporation enhances mass transfer rates, facilitating the release of intra- and inter-cellular compounds (Alexandre, Castro, Moreira, Pintado, & Saraiva, 2017). However, it is very important to find the best operating conditions when PEF treatment is applied before or during food processing.

To optimize the PEF pre-treatment conditions for the extraction of procyanidins from avocado by-products two Box-Behnken designs were performed and are presented in Tables 1 and 2, for the avocado peels and seeds, respectively.

The response evaluated was the sum of procyanidins in both cases. The procyanidins content ranged from 4730.65 to 9202.28 μg CE/g d.w. and from 14094.46 to 20528.02 μg CE/g d.w. in peel (Table 1) and seed (Table 2) models, respectively. The experimental data were fitted to

Table 1
Box Behnken experimental design for the extraction of procyanidins from avocado peel by PEF. Different letters (a-f) indicate significant differences among samples ($p < 0.05$).

N	Frequency (Hz)	Electric field (kV/cm) (V)	Number of pulses	Sum of procyanidins (μg CE/g d.w.)
	X1	X2	X3	
1	1	0.8	150	5355.96 ± 59.23 g
2	1	1.5	150	4730.65 ± 338.34 g
3	100	0.8	150	8611.25 ± 282.99 b-d
4	100	1.5	150	8108.91 ± 337.97 c,d
5	50	0.8	100	4830.52 ± 373.40 g
6	50	1.5	100	6776.80 ± 126.38 e,f
7	50	0.8	200	7185.27 ± 65.78 e
8	50	1.5	200	9682.71 ± 525.30 a
9	1	1.1	100	6055.49 ± 738.28 f
10	100	1.1	100	6474.25 ± 311.03 f
11	1	1.1	200	6326.48 ± 182.87 f
12	100	1.1	200	7984.18 ± 363.01 d
13	50	1.1	150	9202.28 ± 186.81 b
14	50	1.1	150	8938.09 ± 161.52 b
15	50	1.1	150	8709.67 ± 1275.12 b,c

Table 2
Box Behnken experimental design for the extraction of procyanidins from avocado seed by PEF. Different letters (a-e) indicate significant differences among samples ($p < 0.05$).

N	Frequency (Hz)	Electric field (kV/cm)	Total operating time (s)	Sum of procyanidins (μg CE/g d.w.)
	X1	X2	X3	
1	50	0.7	1	15681.11 ± 706.83 d
2	150	0.7	1	15956.48 ± 524.21 c,d
3	50	0.7	10	15864.38 ± 294.85 c,d
4	150	0.7	10	17160.18 ± 648.78 c
5	50	0.6	5	15693.00 ± 180.79 d
6	150	0.6	5	15368.60 ± 380.48 d,e
7	50	0.8	5	15313.34 ± 74.68 d,e
8	150	0.8	5	15624.00 ± 571.97 d
9	100	0.6	1	15549.79 ± 81.50 d
10	100	0.6	10	14094.46 ± 518.85 e
11	100	0.8	1	18994.77 ± 1020.65 b
12	100	0.8	10	20166.46 ± 261.37 a,b
13	100	0.7	5	20236.91 ± 205.84 a,b
14	100	0.7	5	20528.02 ± 332.81 a
15	100	0.7	5	20057.35 ± 237.53 a,b

Table 3
Regression coefficients effect of the avocado peel and seed optimization models.

Regression coefficients	Avocado peel		Avocado seed	
	Effect	p value	Effect	p value
β_0	6931.00[a]	0.0000	16301.72[a]	0.0000
Linear				
β_1	2425.17[a]	0.0000	295.08	0.1576
β_2	431.31[a]	0.0001	1642.04[a]	0.0000
β_3	1341.19[a]	0.0000	415.05[a]	0.0491
Crossed				
β_{12}	−36.74	0.7832	317.53	0.2521
β_{13}	819.28[a]	0.0000	510.21	0.0701
β_{23}	469.78[a]	0.0012	1313.51[a]	0.0000
Quadratic				
β_{11}	1312.81[a]	0.0000	2881.89[a]	0.0000
β_{22}	890.63[a]	0.0000	1846.06[a]	0.0000
β_{33}	831.44[a]	0.0000	1218.78[a]	0.0000
R^2	0.9789		0.9510	
***p* model**	0.0000[a]		0.0001[a]	
***p* lack of fit**	0.7832		0.1035	

[a] Significant $p < 0.05$.

second-order polynomial equations (Equation (1)). Table 3 exhibits the regression coefficients along with their effects and corresponding p values. A significance level of $p < 0.05$ was applied across all cases. Non-significant terms were omitted, leading to the refinement of the models by considering only the significant ones.

In the avocado peel model, all linear terms (β_1, β_2, and β_3), crossed interactions (β_{13} and β_{23}), and quadratic terms (β_{11}, β_{22}, and β_{33}) displayed significant effects on the response variable. As indicated in Table 3, both linear and quadratic terms exhibited positive effects. However, only the crossed term, β_{12} (related to frequency and voltage), displayed a non-significant negative impact on the model. Notably, the frequency factor (β_1) demonstrated the most substantial positive influence, followed by the linear factor representing the number of pulses (β_3). Moreover, a strong correlation between the response variable and the factors was observed ($R^2 = 0.9789$). The ANOVA test confirmed the validity of the model, showing a significant p-value for the model and a non-significant lack of fit ($p > 0.05$).

Regarding the avocado seed model, all quadratic terms were found to have a significant influence. Besides, the linear terms β_2 (corresponding to electric field strength), β_3 (corresponding to total operating time) and the crossed term between them β_{23} were significative ($p < 0.05$). In contrast the linear term corresponding to frequency (β_1) and the rest of interactions (β_{12} and β_{13}) did not have significance in the model. All the variables exhibited positive effect with the quadratic term of frequency (β_{11}) being higher, followed by the quadratic term of electric field (β_{22}). Additionally, a robust relationship was found between the dependent variables and the factors, displaying a high R^2 value of 0.9510. The validity of the model was supported by ANOVA test, indicating a significant p value for the model and a non-significant lack of fit ($p > 0.05$).

3.2. *Optimization of PEF extraction conditions*

The response surface graphs were analyzed to determine the best conditions for each by-product (i.e. peels or seeds), with the aim of achieving a balance between the factors evaluated and obtaining extracts with the maximum total procyanidin content.

Fig. 1 shows that the highest responses for the avocado peel model were obtained at electric fields strength higher than 1.1 kV/cm and a pulse number higher than 150 pulses. In terms of frequency, the highest responses were achieved between 40 and 80 Hz, therefore the lowest frequency possible was selected in order to reduce thermal stress on the treated products as the heating associated with the process is also lower. At lower frequencies, the equipment requires less energy to generate the pulses, which can result in greater energy efficiency and savings in operating costs. Although the amount of energy applied per pulse is the same, the rest time between pulses is longer, which can mean lower overall energy use. The number of pulses was kept as low as possible, considering the low frequency, to achieve a faster treatment time.

Similar responses surfaces were obtained for the avocado seed model as shown in Fig. 2. In this case, the higher responses were achieved at a frequency between 80 and 120 Hz, higher than in the case of the peel model. The best electric field strength appeared to be in the range of 0.7–0.8 kV/cm. Regarding the total treatment time, the intermedium value tested (5 s) showed the best results.

Thus, both models were processed and individually fitted to a quadratic function for predicting the optimum parameters to obtain the higher amount of procyanidins. For this purpose, the responses for each factor were normalized into a dimensionless scale between 0 and 1. This normalization allows for the combination of different factors, despite their different units or scales. It was considered also in combination with response surface methodology.

Briefly, the optimal conditions selected for the avocado peel model were a frequency of 50 Hz, an electric field strength of 1.31 kV/cm and pulse number of 175 with a desirability value of 0.9468 and a predicted procyanidin content of 9845.41 ± 222.60 μg CE/g d.w. Concerning the avocado seed model, a frequency of 100 Hz, a field strength of 0.75 kV/

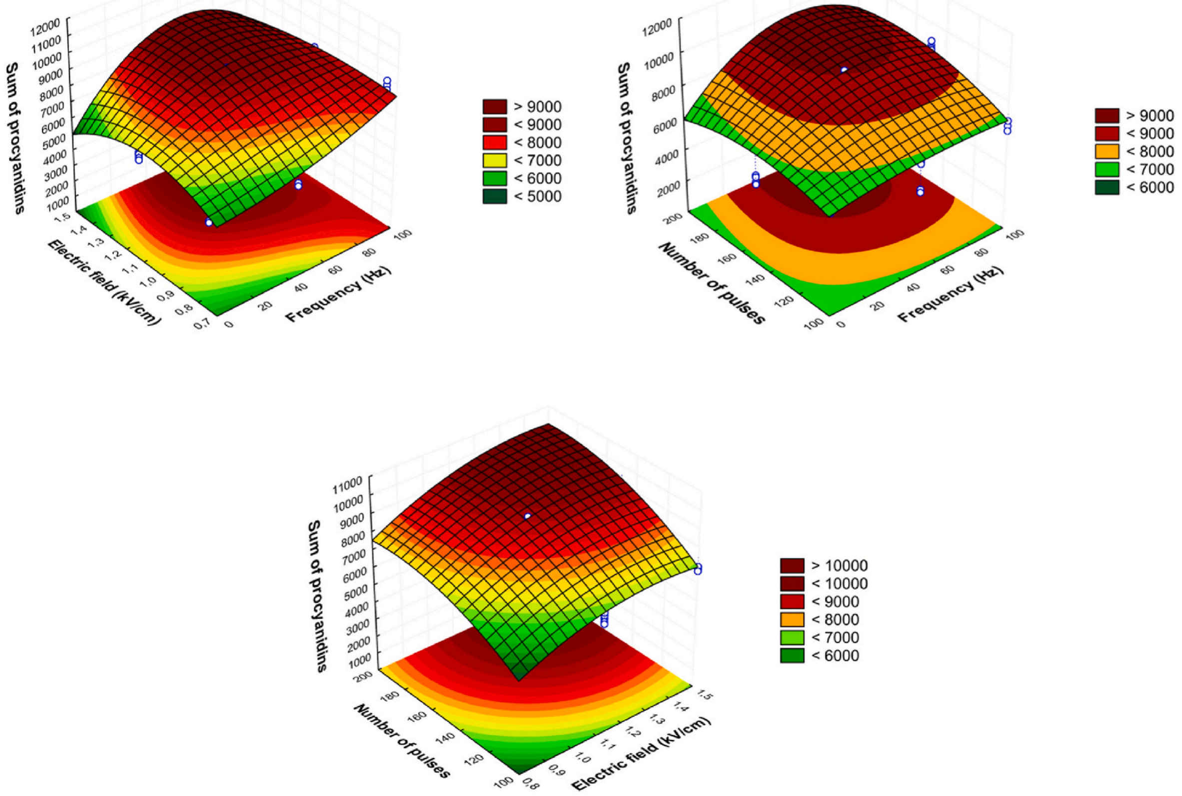

Fig. 1. Response surface plots showing combined effects of process variables for the sum of procyanidins in avocado peel model expressed as μg/g d.w.

cm and a total operating time (i.e. calculated as number of pulses/frequency) of 5.5 s were selected as optimal, having a desirability value of 1 and a predicted procyanidin content of 20955.06 ± 452.35 μg/g d.w. of procyanidins. The accuracy of both mathematical models underwent confirmation (Table 4). In all cases, there were no significant deviations between the values predicted by the model and the actual results obtained under the optimal conditions. Furthermore, the coefficients of variation remained under 1 for both the peel and seed models respect to total procyanidins. Additional supplementary parameters derived from the optimized one are also presented in Table 4.

Several studies have reported the measurement of total phenolic compounds in avocado peels and seeds using various extraction methods such as hydroethanolic liquid-liquid extraction (Melgar et al., 2018), ultrasound bath (Monzón et al., 2021; Tremocoldi et al., 2018), sonotrode (Razola-Díaz, Verardo, et al., 2023), microwave (Carvalho Gualberto et al., 2021) and vacuum microwave (Skenderidis et al., 2021) assisted extractions. However, the quantification was performed by Folin Ciocalteu (spectrophotometric method) or HPLC-MS, not comparable with the results obtained here. Therefore, the optimal extracts were compared to non-PEF treated controls and the results are shown in Fig. 3.

According to the results, a significant ($p < 0.05$) increase of 9.5 % in total procyanidins was achieved in the avocado seeds compared to the control. Regarding the avocado peel it a significant ($p < 0.05$) increment of 31.5% in total procyanidins was achieved compared to control. Previous research have reported results in the range of 4.29–18.74 and 1.05–23.68 mg CE/g d.w. for avocado seed and peel, respectively (López-Cobo et al., 2016; Razola-Díaz, Guerra-Hernández, et al., 2023; Razola-Díaz, Verardo, et al., 2023). The differences in terms of numerical values compared to our previous studies could be due to differences in the ripening stage of avocados, the month of harvesting or minor differences in the processing and the avocado origin. No other previous references were found regarding measuring the procyanidin content by HPLC-FLD in avocado samples. Otherwise, we examined additional studies concerning flava-3-ols analyzed through HPLC-FLD in different matrices. In cocoa powders, the total procyanidin content varied between 3.31 and 28.58 mg/g d.w. (Razola-Díaz, Aznar-Ramos, et al., 2023). *Psidium guajava* leaves exhibited a range of 10.5–15.8 mg/g d.w. (Díaz-de-Cerio et al., 2017), cranberry extracts showed values from 0.73 to 22.29 mg/g d.w. (Wallace & Giusti, 2010), barley flour demonstrated a range of 0.29–0.65 mg/g d.w. (Verardo et al., 2015), and grape seeds displayed content ranging from 3.91 to 14.18 mg/g d.w. (Bombai et al., 2017). Thus, the obtained values are in the same range of magnitude, but none of them applied PEF.

Additionally, the complete procyanidin profile is shown in Table 5.

No significant changes in the procyanidin profile were observed between the optimum and control extracts. Regarding the avocado peel, monomers accounted for 68 and 70% of the total procyanidin content in control and optimum, respectively, and dimers accounted for 27% in both cases. In avocado seed, monomers, dimers, and trimers accounted for 80, 5 and 8%, respectively, in both extracts. All this is in agreement with previous research (Razola-Díaz, Verardo, et al., 2023).

To the best of our knowledge, there are no previous references regarding the use of PEF in avocado by-products for any purposes. The only research regarding PEF and avocado was conducted by Castorena-Garcia et al. (2013) and reported the inactivation up to 85% of polyphenol oxidase enzyme from avocado by using an electric field strength of 18 kV/cm at frequencies above 1000 Hz.

However, there are few references on the use of PEF for improving polyphenol extraction from seeds. Rahmah, Sukardi, and Ahsan (2020) reported an increase of 3.16–14.14% in the tannin content in *Areca catechu* L. seed powder treated with 1.92–12.44 kJ/cm^3 and 6000 Hz. Teh, Niven, Bekhit, Carne, and Birch (2015) used the ratio 1/10 sample/solvent for treating with PEF canola seed cake powder and applied a

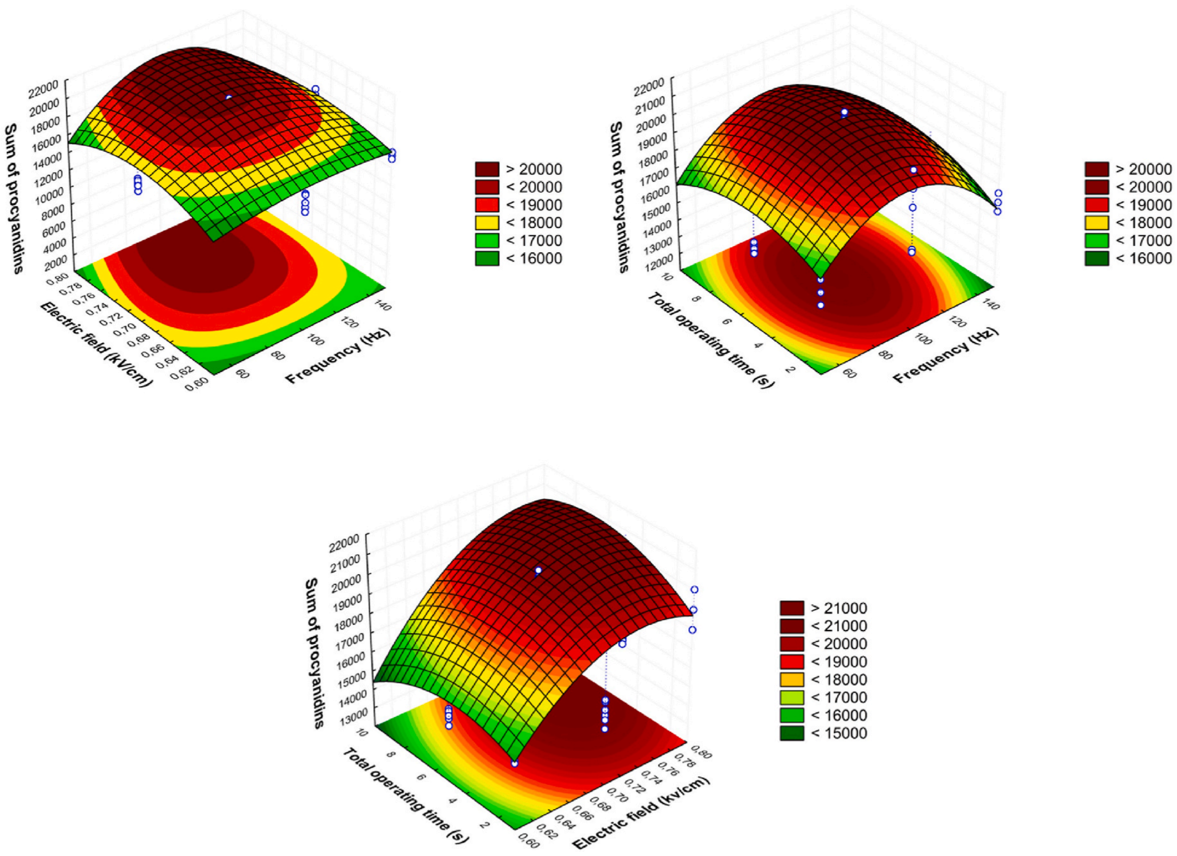

Fig. 2. Response surface plots showing combined effects of process variables for the sum of procyanidins in avocado seed model expressed as μg/g d.w.

Table 4
Optimal conditions of the experimental models for extracting procyanidins from avocado peel and seed by PEF.

	Avocado peel		Avocado seed
Optimized parameters		Optimized parameters	
Frequency (Hz), X_1	50	Frequency (Hz), X_1	100
Field strength (kV/cm), X_2	1.31	Field strength (kV/cm), X_2	0.75
Number of pulses, X_3	175	Total operating time (s), X_3	5.5
Other parameters		**Other parameters**	
Specific energy (kJ/kg)	0.63	Specific energy (kJ/kg)	12.7
Pulse width (μs)	10	Pulse width (μs)	10
Total treatment time (ms)	1.75	Total treatment time (ms)	5.5
Total operating time (s)	3.5	Number of pulses	550
Voltage (V)	2625	Voltage (V)	1350
	Sum of procyanidins		**Sum of procyanidins**
Desirability value	0.9468		1.0000
Predicted value (μg CE/g d.w.)	9845.41 ± 222.60		20955.06 ± 452.35
Obtained value (μg CE/g d.w.)	9798.32 ± 103.83		21103.12 ± 234.76
CV (%) Predicted vs. Obtained	0.34		0.50
Control (μg CE/g d.w.)	6710.59 ± 219.28		19103.39 ± 172.76

CE: Catechin equivalents; CV: Coefficient of variation. Predicted, obtained and control sum of procyanidin values are expressed as average ± standard deviation.

frequency of 30 Hz and 1.1 kV/cm with a total operating time of 10 s as optimal parameter for extracting polyphenols. Parniakov et al. (2015) PEF treated a suspension of powdered papaya seeds at a ratio solid/liquid of 1/10 using an electric field of 13.3 kV/cm and a total operating time of 2000 s for the extraction of phenolic compounds. Boussetta, Vorobiev, Le, Cordin-Falcimaigne, and Lanoisellé (2012) treated grape seeds, with a particle size of 4 mm, with a PEF at 20 kV/cm with a frequency of 0.33 Hz for a total treatment time of 20 ms increasing the polyphenol extraction. Boussetta, Soichi, Lanoisellé, and Vorobiev (2014) applied PEF to flaxseed hulls at 20 kV/cm for 10 ms also with improvements in the polyphenol extraction. Thus, the obtained optimal parameters are in the range of values reported in other samples.

PEF has been reported previously by other authors to improve the extraction of polyphenols in other peels such as in papaya peels (Parniakov et al., 2014), grape peels (Medina-Meza & Barbosa-Cánovas, 2015) and pomegranate peels (Rajha et al., 2019). Shorstkii et al. (2023) reported an increase in the phenolic content of kiwifruit peel by 5.1% by using a PEF treatment at 100 V/cm and 0.96 kJ/kg. Frontuto et al. (2019) used PEF as a pre-treatment for extracting phenolic compounds from potato peels with an increase of 10% in the yield using 1 kV/cm and 5 kJ/kg. Pataro et al. (2018) reported an increase of 188% in the recovery of carotenoids from tomato peels by using PEF at a specific energy of 1 kJ/kg, electric field strength of 0.75 kV/cm, frequency of 10 Hz and pulse width of 20 μs. Pataro, Carullo, Falcone, and Ferrari (2020) reported an increase of 12–18% in the lycopene content in tomato peel by using PEF treatment at 5 kV/cm and 5 kJ/kg. Peiró, Luengo, Segovia, Raso, and Almajano (2019) improved the extraction of polyphenols from lemon peels using a PEF pre-treatment at 7 kV/cm and 1 Hz. Redondo, Venturini, Luengo, Raso, and Arias (2018) identified the optimal condition of PEF treatment (5 kV/cm, 5.98 kJ/kg and 1 Hz) to improve the phenolic compounds recovery from the peach fruit peel. In orange peels, Luengo et al. (2013) reported that a PEF treatment (7 kV/cm, 3.77 kJ/kg, 1 Hz) increased the antioxidant activity of the extracts by 192%. In our study, the optimal parameters determined are within the range of the values reported in literature.

In this context, it should be mentioned that the avocado seed

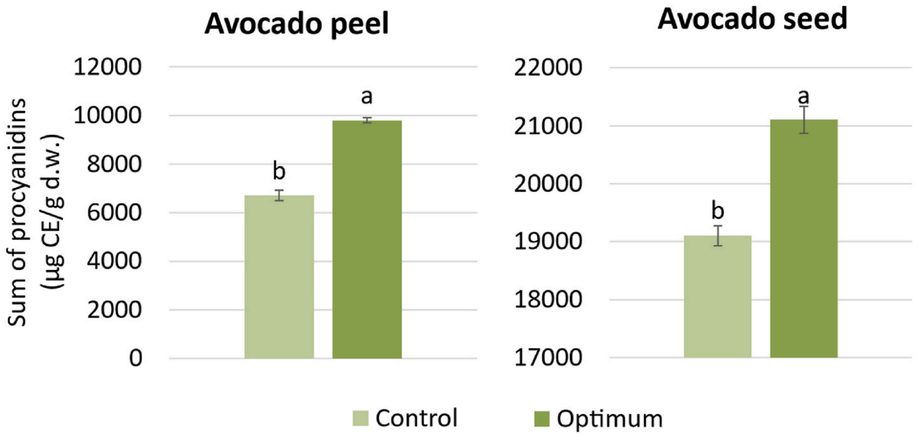

Fig. 3. Sum of procyanidins of optimum PEF pre-treated extracts and the controls (not treated) for avocado peels and seeds, expressed as µg catechin equivalents (CE)/g d.w. Different letters (a,b) for each sample indicate significant differences ($p < 0.05$).

Table 5
Procyanidin distribution content of procyanidin in avocado peel and seed extract obtained using the optimal PEF conditions and the controls measured by HPLD-FLD-DAD and expressed in µg CE/g d.w. with average ± standard deviation.

	Avocado peel		Avocado seed	
	Control	Optimum	Control	Optimum
Monomers (cat + epicat)	4721.58 ± 101.69 d	6673.37 ± 70.72 c	15475.72 ± 86.12 b	16975.65 ± 188.84 a
Dimers	1849.67 ± 75.03 b	2735.55 ± 28.99 a	980.04 ± 32.56 d	1245.75 ± 13.86 c
DP3	58.55 ± 19.94 d	172.52 ± 1.83 c	1559.39 ± 46.67 b	1683.27 ± 18.73 a
DP4	35.13 ± 10.09 d	96.77 ± 1.03 c	407.70 ± 7.46 b	453.12 ± 5.04 a
DP5	21.86 ± 6.13 d	58.32 ± 0.62 c	242.85 ± 3.91 b	267.08 ± 2.97 a
DP6	11.05 ± 3.10 d	28.58 ± 0.30 c	135.35 ± 1.04 b	145.78 ± 1.62 a
DP7	5.49 ± 1.44 d	15.48 ± 0.16 c	92.71 ± 1.69 b	101.26 ± 1.13 a
DP8	3.05 ± 0.55 d	6.30 ± 0.07 c	59.45 ± 1.01 b	64.49 ± 0.72 a
DP9	1.82 ± 0.01 d	3.62 ± 0.04 c	38.20 ± 0.04 b	39.77 ± 0.44 a
DP10	1.64 ± 0.24 c	2.66 ± 0.03 b	27.66 ± 0.28 a	27.85 ± 0.31 a
DP11	< LOD	3.49 ± 0.04 b	26.34 ± 0.30 a	26.52 ± 0.30 a
DP12	< LOD	< LOD	25.86 ± 0.05 a	25.63 ± 0.29 a
Polymers	0.74 ± 0.05 c	1.65 ± 0.02 c	32.12 ± 7.02 b	46.94 ± 0.52 a

Cat + epicat: catechin + epicatechin; DP: degree of polymerization. Different letters (a-d) in the same line indicate significant differences ($p < 0.05$).

required a higher specific energy (12.7 kJ/kg versus 0.63 kJ/kg in the avocado peel), a higher number of pulses (550 versus 175 in the avocado peel) and, consequently a longer treatment (5.5 ms versus 1.75 ms) and operating time (5.5 s versus of 3.5 s). In contrast, the applied electric field strength was higher for the peel (1.31 kV/cm) than for the seed (0.75 kV/cm). This could be attributed to the differences between the sample's structures and kind of cells. Avocado seed have more lignin making it more difficult to electroporate. In the case of avocado peel, pieces of 2–3 cm were used, while in the case of avocado seed it was created a paste in ratio 1:2 seed/water with milled seed of a size of 0.2–5 µm. It is a fact that the extent of electroporation relies on various factors including plant material compounds, cell physiology, electric field intensity, pulse waveform, treatment duration, number of pulses. This selective extraction process yields extracts of higher purity and increased extraction efficiency. Although the exact mechanisms of electroporation are not yet fully understood, it is evident that the membrane plays a pivotal role in amplifying the applied electric field. This is particularly significant due to the considerable difference in conductivity between intact membranes and the extracellular medium or cell cytoplasm (Alexandre et al., 2017).

4. Conclusions

The results of the study underline the effectiveness of pulsed electric fields (PEF) as a promising technology for procyanidin extraction from avocado peels and seeds. Optimized conditions, including a frequency of 50 Hz, electric field strength of 1.31 kV/cm, and 175 pulses for the avocado peel, and a frequency of 100 Hz, electric field strength of 0.75 kV/cm, and total operating time of 5.5 s for the avocado seeds, resulted in substantial increase in procyanidin content. The procyanidin enhancement achieved— + 31.5% in avocado peels and +9.5% in avocado seeds—compared to non-PEF treated control samples, demonstrates the effectiveness of the selective extraction process. The findings align with market trends for natural-source polyphenols, presenting a practical and scalable solution for industries seeking sustainable practices. Furthermore, the study's rigorous validation of mathematical models ensures the reliability of the proposed optimized conditions, paving the way for immediate applications in the fruit and vegetable processing industry. This research contributes to a paradigm shift in avocado waste management, emphasizing resource efficiency, environmental responsibility, and economic viability through innovative extraction technologies.

CRediT authorship contribution statement

María del Carmen Razola-Díaz: Writing – original draft, Investigation, Formal analysis. **Jessica Genovese:** Writing – review & editing, Formal analysis, Data curation. **Urszula Tylewicz:** Writing – review & editing, Methodology. **Eduardo J. Guerra-Hernández:** Writing – review & editing, Supervision, Funding acquisition. **Pietro Rocculi:** Writing – review & editing, Supervision. **Vito Verardo:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

All data are in the manuscript

References

Alexandre, E. M. C., Castro, L. M. G., Moreira, S. A., Pintado, M., & Saraiva, J. A. (2017). Comparison of emerging technologies to extract high-added value compounds from fruit residues: Pressure- and electro-based technologies. *Food Engineering Reviews, 9*(3), 190–212. https://doi.org/10.1007/s12393-016-9154-2

Araujo, R. G., Rodríguez-Jasso, R. M., Ruíz, H. A., Govea-Salas, M., Pintado, M., & Aguilar, C. N. (2021). Recovery of bioactive components from avocado peels using microwave-assisted extraction. *Food and Bioproducts Processing, 127*, 152–161. https://doi.org/10.1016/j.fbp.2021.02.015

Araújo, R. G., Rodriguez-Jasso, R. M., Ruiz, H. A., Pintado, M. M. E., & Aguilar, C. N. (2018). Avocado by-products: Nutritional and functional properties. *Trends in Food Science & Technology, 80*(August), 51–60. https://doi.org/10.1016/j.tifs.2018.07.027

Arshad, R. N., Abdul-Malek, Z., Roobab, U., Munir, M. A., Naderipour, A., Qureshi, M. I., et al. (2021). Pulsed electric field: A potential alternative towards a sustainable food processing. *Trends in Food Science & Technology, 111*(May), 43–54. https://doi.org/10.1016/j.tifs.2021.02.041

Barba, F. J., Brianceau, S., Turk, M., Boussetta, N., & Vorobiev, E. (2015). Effect of alternative physical treatments (ultrasounds, pulsed electric fields, and high-voltage electrical discharges) on selective recovery of bio-compounds from fermented grape pomace. *Food and Bioprocess Technology, 8*(5), 1139–1148. https://doi.org/10.1007/s11947-015-1482-3

Bobinaitė, R., Pataro, G., Lamanauskas, N., Šatkauskas, S., Viškelis, P., & Ferrari, G. (2015). Application of pulsed electric field in the production of juice and extraction of bioactive compounds from blueberry fruits and their by-products. *Journal of Food Science and Technology, 52*(9), 5898–5905. https://doi.org/10.1007/s13197-014-1668-0

Bombai, G., Pasini, F., Verardo, V., Sevindik, O., Di Foggia, M., Tessarin, P., et al. (2017). Monitoring of compositional changes during berry ripening in grape seed extracts of cv. Sangiovese (Vitis vinifera L.). *Journal of the Science of Food and Agriculture, 97*(9), 3058–3064. https://doi.org/10.1002/jsfa.8151

Boussetta, N., Soichi, E., Lanoisellé, J.-L., & Vorobiev, E. (2014). Valorization of oilseed residues: Extraction of polyphenols from flaxseed hulls by pulsed electric fields. *Industrial Crops and Products, 52*, 347–353. https://doi.org/10.1016/j.indcrop.2013.10.048

Boussetta, N., Vorobiev, E., Le, L. H., Cordin-Falcimaigne, A., & Lanoisellé, J.-L. (2012). Application of electrical treatments in alcoholic solvent for polyphenols extraction from grape seeds. *LWT - Food Science and Technology, 46*(1), 127–134. https://doi.org/10.1016/j.lwt.2011.10.016

Brianceau, S., Turk, M., Vitrac, X., & Vorobiev, E. (2015). Combined densification and pulsed electric field treatment for selective polyphenols recovery from fermented grape pomace. *Innovative Food Science & Emerging Technologies, 29*, 2–8. https://doi.org/10.1016/j.ifset.2014.07.010

Carvalho Gualberto, N., Santos de Oliveira, C., Pedreira Nogueira, J., Silva de Jesus, M., Caroline Santos Araujo, H., Rajan, M., et al. (2021). Bioactive compounds and antioxidant activities in the agro-industrial residues of acerola (Malpighia emarginata L.), guava (Psidium guajava L.), genipap (Genipa americana L.) and umbu (Spondias tuberosa L.) fruits assisted by ultrasonic or shaker extracti. *Food Research International, 147*(March), Article 110538. https://doi.org/10.1016/j.foodres.2021.110538

Castorena-Garcia, J. H., Martinez-Montes, F. J., Robles-Lopez, M. R., Welti-Chanes, J. S., Hernandez-Sanchez, H., & Robles-de-la-Torre, R. R. (2013). Effect of electric fields on the activity of polyphenol oxidases. *Revista Mexicana de Ingenieria Quimica, 12*(3), 391–400. http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S1665-27382013000300003&lng=es&tlng=en.

Chakka, A. K., Sriraksha, M. S., & Ravishankar, C. N. (2021). Sustainability of emerging green non-thermal technologies in the food industry with food safety perspective: A review. *LWT, 151*(December 2020), Article 112140. https://doi.org/10.1016/j.lwt.2021.112140

Cheng, J. Y. K., Chiu, S. L. H., & Lo, I. M. C. (2017). Effects of moisture content of food waste on residue separation, larval growth and larval survival in black soldier fly bioconversion. *Waste Management, 67*, 315–323. https://doi.org/10.1016/j.wasman.2017.05.046

Corrales, M., Toepfl, S., Butz, P., Knorr, D., & Tauscher, B. (2008). Extraction of anthocyanins from grape by-products assisted by ultrasonics, high hydrostatic pressure or pulsed electric fields: A comparison. *Innovative Food Science & Emerging Technologies, 9*(1), 85–91. https://doi.org/10.1016/j.ifset.2007.06.002

Díaz-de-Cerio, E., Pasini, F., Verardo, V., Fernández-Gutiérrez, A., Segura-Carretero, A., & Caboni, M. F. (2017). Psidium guajava L. leaves as source of proanthocyanidins: Optimization of the extraction method by RSM and study of the degree of polymerization by NP-HPLC-FLD-ESI-MS. *Journal of Pharmaceutical and Biomedical Analysis, 133*, 1–7. https://doi.org/10.1016/j.jpba.2016.10.011

Frontuto, D., Carullo, D., Harrison, S. M., Brunton, N. P., Ferrari, G., Lyng, J. G., et al. (2019). Optimization of pulsed electric fields-assisted extraction of polyphenols from potato peels using response surface methodology. *Food and Bioprocess Technology, 12*(10), 1708–1720. https://doi.org/10.1007/s11947-019-02320-z

Galanakis, C. M. (2012). Recovery of high added-value components from food wastes: Conventional, emerging technologies and commercialized applications. *Trends in Food Science & Technology, 26*(2), 68–87. https://doi.org/10.1016/j.tifs.2012.03.003

Galanakis, C. M. (2013). Emerging technologies for the production of nutraceuticals from agricultural by-products: A viewpoint of opportunities and challenges. *Food and Bioproducts Processing, 91*(4), 575–579. https://doi.org/10.1016/j.fbp.2013.01.004

García-Vargas, M. C., Contreras, M. del M., & Castro, E. (2020). Avocado-derived biomass as a source of bioenergy and bioproducts. *Applied Sciences, 10*(22), 8195. https://doi.org/10.3390/app10228195

Hollands, W. J., Voorspoels, S., Jacobs, G., Aaby, K., Meisland, A., Garcia-Villalba, R., et al. (2017). Development, validation and evaluation of an analytical method for the determination of monomeric and oligomeric procyanidins in apple extracts. *Journal of Chromatography A, 1495*, 46–56. https://doi.org/10.1016/j.chroma.2017.03.030

Jimenez, P., Garcia, P., Quitral, V., Vasquez, K., Parra-Ruiz, C., Reyes-Farias, M., et al. (2021). Pulp, leaf, peel and seed of avocado fruit: A review of bioactive compounds and healthy benefits. *Food Reviews International, 37*(6), 619–655. https://doi.org/10.1080/87559129.2020.1717520

López-Cobo, A., Gómez-Caravaca, A. M., Pasini, F., Caboni, M. F., Segura-Carretero, A., & Fernández-Gutiérrez, A. (2016). HPLC-DAD-ESI-QTOF-MS and HPLC-FLD-MS as valuable tools for the determination of phenolic and other polar compounds in the edible part and by-products of avocado. *LWT - Food Science and Technology, 73*, 505–513. https://doi.org/10.1016/j.lwt.2016.06.049

Luengo, E., Álvarez, I., & Raso, J. (2013). Improving the pressing extraction of polyphenols of orange peel by pulsed electric fields. *Innovative Food Science and Emerging Technologies, 17*, 79–84. https://doi.org/10.1016/j.ifset.2012.10.005

Martín-García, B., Tylewicz, U., Verardo, V., Pasini, F., Gómez-Caravaca, A. M., Caboni, M. F., et al. (2020). Pulsed electric field (PEF) as pre-treatment to improve the phenolic compounds recovery from brewers' spent grains. *Innovative Food Science and Emerging Technologies, 64*(October 2019), Article 102402. https://doi.org/10.1016/j.ifset.2020.102402

Mazyan, W. I., O'connor, E., Martin, E., Vogt, A., Charter, E., & Ahmadi, A. (2021). Effects of temperature and extraction time on avocado flesh (Persea americana) total phenolic yields using subcritical water extraction. *Processes, 9*(1), 1–13. https://doi.org/10.3390/PR9010159

Medina-Meza, I. G., & Barbosa-Cánovas, G. V. (2015). Assisted extraction of bioactive compounds from plum and grape peels by ultrasonics and pulsed electric fields. *Journal of Food Engineering, 166*, 268–275. https://doi.org/10.1016/j.jfoodeng.2015.06.012

Melgar, B., Dias, M. I., Ciric, A., Sokovic, M., Garcia-Castello, E. M., Rodriguez-Lopez, A. D., et al. (2018). Bioactive characterization of Persea americana Mill. By-products: A rich source of inherent antioxidants. *Industrial Crops and Products, 111*(October 2017), 212–218. https://doi.org/10.1016/j.indcrop.2017.10.024

Monzón, L., Becerra, G., Aguirre, E., Rodríguez, G., & Villanueva, E. (2021). Ultrasound-assisted extraction of polyphenols from avocado residues: Modeling and optimization using response surface methodology and artificial neural networks. *Scientia Agropecuaria, 12*(1), 33–40. https://doi.org/10.17268/SCI.AGROPECU.2021.004

Ntourtoglou, G., Drosou, F., Chatzimitakos, T., Athanasiadis, V., Bozinou, E., Dourtoglou, V. G., et al. (2022). Combination of pulsed electric field and ultrasound in the extraction of polyphenols and volatile compounds from grape stems. *Applied Sciences, 12*(12). https://doi.org/10.3390/app12126219

Parniakov, O., Barba, F. J., Grimi, N., Lebovka, N., & Vorobiev, E. (2014). Impact of pulsed electric fields and high voltage electrical discharges on extraction of high-added value compounds from papaya peels. *Food Research International, 65*(PC), 337–343. https://doi.org/10.1016/j.foodres.2014.09.015

Parniakov, O., Barba, F. J., Grimi, N., Lebovka, N., & Vorobiev, E. (2016). Extraction assisted by pulsed electric energy as a potential tool for green and sustainable recovery of nutritionally valuable compounds from mango peels. *Food Chemistry, 192*, 842–848. https://doi.org/10.1016/j.foodchem.2015.07.096

Parniakov, O., Roselló-Soto, E., Barba, F. J., Grimi, N., Lebovka, N., & Vorobiev, E. (2015). New approaches for the effective valorization of papaya seeds: Extraction of proteins, phenolic compounds, carbohydrates, and isothiocyanates assisted by pulsed electric energy. *Food Research International, 77*, 711–717. https://doi.org/10.1016/j.foodres.2015.03.031

Pataro, G., Carullo, D., Bakar Siddique, M. A., Falcone, M., Donsì, F., & Ferrari, G. (2018). Improved extractability of carotenoids from tomato peels as side benefits of PEF treatment of tomato fruit for more energy-efficient steam-assisted peeling. *Journal of Food Engineering, 233*, 65–73. https://doi.org/10.1016/j.jfoodeng.2018.03.029

Pataro, G., Carullo, D., Falcone, M., & Ferrari, G. (2020). Recovery of lycopene from industrially derived tomato processing by-products by pulsed electric fields-assisted extraction. *Innovative Food Science and Emerging Technologies, 63*(February), Article 102369. https://doi.org/10.1016/j.ifset.2020.102369

Peiró, S., Luengo, E., Segovia, F., Raso, J., & Almajano, M. P. (2019). Improving polyphenol extraction from lemon residues by pulsed electric fields. *Waste and Biomass Valorization, 10*(4), 889–897. https://doi.org/10.1007/s12649-017-0116-6

Rahmah, N. L., Sukardi, & Ahsan, A. M. (2020). Analysis of pulsed electric field (PEF) specific input energy and its effect to the tannin content of Areca (Areca catechu L.) seed powder extract. *IOP Conference Series: Earth and Environmental Science, 475*(1). https://doi.org/10.1088/1755-1315/475/1/012031

Rajha, H. N., Abi-Khattar, A. M., El Kantar, S., Boussetta, N., Lebovka, N., Maroun, R. G., et al. (2019). Comparison of aqueous extraction efficiency and biological activities of polyphenols from pomegranate peels assisted by infrared, ultrasound, pulsed electric fields and high-voltage electrical discharges. *Innovative Food Science and Emerging Technologies, 58*. https://doi.org/10.1016/J.IFSET.2019.102212

Raso, J., Frey, W., Ferrari, G., Pataro, G., Knorr, D., Teissie, J., et al. (2016). Recommendations guidelines on the key information to be reported in studies of application of PEF technology in food and biotechnological processes. *Innovative Food Science and Emerging Technologies, 37*, 312–321. https://doi.org/10.1016/j.ifset.2016.08.003

Razola-Díaz, M. del C., Aznar-Ramos, M. J., Verardo, V., Melgar-Locatelli, S., Castilla-Ortega, E., & Rodríguez-Pérez, C. (2023). Exploring the nutritional composition and bioactive compounds in different cocoa powders. *Antioxidants, 12*(3), 716. https://doi.org/10.3390/antiox12030716

Razola-Díaz, M. del C., Guerra-Hernández, E. J., Gómez-Caravaca, A. M., García-Villanova, B., & Verardo, V. (2023). Mathematical modelling of drying kinetics of avocado peels and its influence on flavan-3-ols content and antioxidant activity. *LWT, 176*(January), Article 114552. https://doi.org/10.1016/j.lwt.2023.114552

Razola-Díaz, M. del C., Tylewicz, U., Rocculi, P., & Verardo, V. (2023). Application of pulsed electric field processing in the food industry. In *Non-thermal food processing operations* (pp. 257–298). Elsevier. https://doi.org/10.1016/B978-0-12-818717-3.00009-3.

Razola-Díaz, M. del C., Verardo, V., Guerra-Hernández, E. J., García-Villanova Ruiz, B., & Gómez-Caravaca, A. M. (2023). Response surface methodology for the optimization of flavan-3-ols extraction from avocado by-products via sonotrode ultrasound-assisted extraction. *Antioxidants, 12*(7), 1409. https://doi.org/10.3390/antiox12071409

Redondo, D., Venturini, M. E., Luengo, E., Raso, J., & Arias, E. (2018). Pulsed electric fields as a green technology for the extraction of bioactive compounds from thinned peach by-products. *Innovative Food Science and Emerging Technologies, 45*, 335–343. https://doi.org/10.1016/J.IFSET.2017.12.004

Restrepo-Serna, D. L., Solarte-Toro, J. C., & Cardona-Alzate, C. A. (2022). A biorefinery approach for an integral valorisation of avocado peel and seeds through supercritical fluids. *Waste and Biomass Valorization, 13*(9), 3973–3988. https://doi.org/10.1007/s12649-022-01829-3

Rifna, E. J., Misra, N. N., & Dwivedi, M. (2023). Recent advances in extraction technologies for recovery of bioactive compounds derived from fruit and vegetable waste peels: A review. *Critical Reviews in Food Science and Nutrition, 63*(6), 719–752. https://doi.org/10.1080/10408398.2021.1952923

Roselló-Soto, E., Barba, F. J., Parniakov, O., Galanakis, C. M., Lebovka, N., Grimi, N., et al. (2015). High voltage electrical discharges, pulsed electric field, and ultrasound assisted extraction of protein and phenolic compounds from olive kernel. *Food and Bioprocess Technology, 8*(4), 885–894. https://doi.org/10.1007/s11947-014-1456-x

Sandoval-Contreras, T., González Chávez, F., Poonia, A., Iñiguez-Moreno, M., & Aguirre-Güitrón, L. (2023). Avocado waste biorefinery: Towards sustainable development. *Recycling, 8*(5), 81. https://doi.org/10.3390/recycling8050081

Segovia, F. J., Corral-Pérez, J. J., & Almajano, M. P. (2016). Avocado seed: Modeling extraction of bioactive compounds. *Industrial Crops and Products, 85*, 213–220. https://doi.org/10.1016/j.indcrop.2016.03.005

Shorstkii, I., Stuehmeier-Niehe, C., Sosnin, M., Mounassar, E. H. A., Comiotto-Alles, M., Siemer, C., et al. (2023). Pulsed electric field treatment application to improve product yield and efficiency of bioactive compounds through extraction from peels in kiwifruit processing. *Journal of Food Processing and Preservation*, 1–11. https://doi.org/10.1155/2023/8172255, 2023.

Skenderidis, P., Leontopoulos, S., Petrotos, K., & Giavasis, I. (2021). Vacuum microwave-assisted aqueous extraction of polyphenolic compounds from avocado (Persea americana) solid waste. *Sustainability, 13*(4), 1–18. https://doi.org/10.3390/su13042166

Teh, S. S., Niven, B. E., Bekhit, A. E. D. A., Carne, A., & Birch, E. J. (2015). Microwave and pulsed electric field assisted extractions of polyphenols from defatted canola seed cake. *International Journal of Food Science and Technology, 50*(5), 1109–1115. https://doi.org/10.1111/IJFS.12749

Tremocoldi, M. A., Rosalen, P. L., Franchin, M., Massarioli, A. P., Denny, C., Daiuto, É. R., et al. (2018). Exploration of avocado by-products as natural sources of bioactive compounds. *PLoS One, 13*(2), 1–12. https://doi.org/10.1371/journal.pone.0192577

Tylewicz, U. (2020). How does pulsed electric field work? In F. Barba, O. Parniakov, & A. Wiktor (Eds.), *Pulsed electric fields to obtain healthier and sustainable food for tomorrow* (pp. 3–21). Elsevier.

Tzima, K., Brunton, N. P., Lyng, J. G., Frontuto, D., & Rai, D. K. (2021). The effect of Pulsed Electric Field as a pre-treatment step in Ultrasound Assisted Extraction of phenolic compounds from fresh rosemary and thyme by-products. *Innovative Food Science and Emerging Technologies, 69*(January), Article 102644. https://doi.org/10.1016/j.ifset.2021.102644

Verardo, V., Cevoli, C., Pasini, F., Gómez-Caravaca, A. M., Marconi, E., Fabbri, A., et al. (2015). Analysis of oligomer proanthocyanidins in different barley genotypes using high-performance liquid chromatography-fluorescence detection-mass spectrometry and near-infrared methodologies. *Journal of Agricultural and Food Chemistry, 63*(16), 4130–4137. https://doi.org/10.1021/acs.jafc.5b01425

Wallace, T. C., & Giusti, M. M. (2010). Extraction and normal-phase HPLC-fluorescence-electrospray MS characterization and quantification of procyanidins in cranberry extracts. *Journal of Food Science, 75*(8), 690–696. https://doi.org/10.1111/j.1750-3841.2010.01799.x